Cellular Physiology and Biochemistry

Cell Physiol Biochem 2018;47:1399-1410
DOI: 10.1159/000490829
Published online: June 22, 2018
Accepted: April 13, 2018

Original Paper

Induction of Pro-Inflammatory Response via Activated Macrophage-Mediated NF-κB and STAT3 Pathways in Gastric Cancer Cells

Yujuan Zhou Longzheng Xia Qiang Liu Heran Wang Jingguan Lin Linda Oyang Xiaoyan Chen Xia Luo Shiming Tan Yutong Tian Min Su Ying Wang Pan Chen Yang Wu Hui Wang Qianjin Liao

Hunan Key Laboratory of Translational Radiation Oncology, Hunan Cancer Hospital and The Affiliated Cancer Hospital of Xiangya School of Medicine, Central South University, Changsha, China

Key Words
Gastric cancer • Macrophage • Inflammation • Nuclear factor-kappa B • Signal transducer and activator of transcription 3

Abstract
Background/Aims: Chronic inflammation plays an important role in the initiation and progression of gastric cancer (GC). However, the role and relationship of activated macrophages with gastric mucous epithelium cells in initiating and maintaining the inflammatory process during gastric carcinogenesis remains unclear. ***Methods:*** The tumour associated macrophages (TAMs) density of gastric cancer was characterized by immunohistochemistry, and the relationship between macrophages and gastric epithelium cells was analysed using an *in vitro* culture system that imitates the inflammatory microenvironment. The production of pro-inflammatory cytokines was detected by enzyme-linked immunosorbent assay (ELISA) and quantitative real-time PCR (qRT-PCR). MTT assays, Western blotting, qRT-PCR, and luciferase reporter assays were used to detect the effects of cell proliferation, as well as the NF-κB and STAT3 signalling pathways. ***Results:*** TAMs infiltrated with a high intensity in GC and were significantly correlated with histology grade (P = 0.012), metastasis (P = 0.001), TNM stage (P = 0.002), and poor prognosis in patients (PFS, P = 0.005; OS, P = 0.028). In addition, IL-6 and IL-8 were elevated in the serum of GC patients and significantly promoted the growth of GC. The exposure of BGC823 gastric cancer cells to a conditioned medium from LPS-treated D-THP-1 cells significantly induced the production of TNF-α, IL-6, IL-1β and IL-8 (P< 0.01). LPS and LPS-treated D-THP-1-conditioned media promoted gastric cancer cell proliferation and triggered the significant activation of NF-κB and STAT3 with a concomitant degradation of IκBα and an

Y. Zhou and L. Xia contributed equally to this work.

Qianjin Liao
and Hui Wang

Hunan Key Lab. of Transl. Radiation Oncology, Hunan Cancer Hosp. and The Aff. Cancer Hosp. of Xiangya, School of Med., Central South Univ., 283 Tongzipo Road, Changsha 410013, Hunan (China); E-Mail march-on@126.com, wanghui710327@163.com

increase in JAK2 phosphorylation ($P < 0.05$). Moreover, gastric cancer cells markedly expressed cell membrane LPS receptors, such as TLR1, TLR4, TLR6, CD14 and MD2. ***Conclusions:*** TAMs are closely associated with the growth of GC and prognosis in GC patients. GC cells may directly sustain and amplify the local pro-inflammatory response upon encountering activated macrophages and LPS via NF-κB and STAT3 signalling pathways, thereby promoting tumour progression.

Introduction

Gastric cancer (GC) is the second most common cause of cancer-related deaths world wide [1], and it is also the second leading cause of cancer mortality in China [1]. However, the pathogenic mechanism of GC is still not clear. In recent years, attention to the "inflammation to cancer chain" has suggested that most gastric cancers are caused by chronic inflammation in gastric mucosa [2], especially helicobacter pylori (HP) infected gastritis [3-5]. Helicobacter pylori bacteria have been thought to be initiators of the Correa cascade fromchronic reactive gastritis to chronic atrophic gastritis, intestinal metaplasia, atypical hyperplasia and ultimately gastric cancer [4, 6]. Lipopolysaccharide (LPS) binds to external receptors and activates cellular signal pathways, promoting the occurrence and development of GC [6]. In addition, EBV infection can also promote development of GC [7, 8]. Therefore, GC is considered an inflammation-related cancer [2, 9]. Understanding the mechanism of the chronic inflammation leading to GC would provide a new therapeutic strategy for GC. Although it has been increasingly reported that chronic inflammation plays a role in gastric carcinogenesis, it is still unclear how the gastric mucous epithelium cells are involved in the inflammatory process that results in chronic inflammation, especially the complex interaction of GC cells with their microenvironment.

It is well known that the microenvironment of solid tumours is rich in inflammatory cells that influence tumour growth and development [10]. Macrophages, especially so-called tumour-associated macrophages (TAMs), are the most abundant immune cell population present in tumour tissues, and they are usually marked by CD68 [11, 12]. The polarization of macrophages into tumour-suppressive M1 or tumour-promoting M2 types is a fundamental event in the establishment of the tumour microenvironment, and ample evidence has indicated that TAMs are primarily M2 polarized, which act as pro-tumourous factors in many types of human tumours [13, 14]. iNOS and CD163 are the markers of M1 and M2, respectively [15]. It is well known that TAMs typically promote cancer cell proliferation, immunosuppression, and angiogenesis in support of tumour growth and metastasis [16, 17]. Numerous studies have shown that cancer tissues with high infiltration of TAMs are associated with resistance to therapies and poor patient prognosis [18, 19]. However, the function and mechanism of TAMs in GC remain unclear. Clarifying the roles of activated macrophages or other immune cells and the interaction with gastric mucous epithelium cells during chronic inflammation will contribute to an understanding of how the pro-inflammatory response is initiated and amplified during gastric carcinogenesis.

In this study, we examined the infiltration of TAMs in gastric cancer tissues and the clinical significance, and we assessed the response of gastric carcinoma epithelial cells to LPS and TAMs through simulating an inflammation microenvironment *in vitro*. Our results provided evidence and clarified the role and relationship of TAMs with gastric mucous epithelial cells in initiating and maintaining the inflammation process during gastric carcinogenesis.

Materials and Methods

Patient samples

To study the macrophage infiltrated density, a total of 90 surgically resected gastric cancer specimens were collected from 2008 to 2010 at our hospital. The specimens were collected from patients who did not